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known as SEQ ID No. 4, encoded by a nucleic acid molecule encoding said growth factor [or a functional equivalent, derivative or bioprecursor of said growth factor].

8. (Twice amended) A growth factor according to claim 7 comprising the amino acid sequence from position 27 to 139 of the amino acid sequence illustrated in Figure 1, [as] known as SEQ ID No. 3[, or a functional equivalent, derivative or bioprecursor of said growth factor].

9. (Twice amended) A growth factor according to claim [7] 8 comprising the amino acid sequence illustrated in Figure 1, [as] known as SEQ ID No. 4[, or a functional equivalent, derivative or bioprecursor of said growth factor].

REMARKS

Claims 7-9, 17-18, 24, 41, 44, and 58-59 are pending in this application. The Examiner has:

(1) objected to the specification;

(2) withdrew claims 17-18, 24, 41, 44, and 58-59 from further consideration as being directed to a non-elected invention;

(3) objected to claims 7-9 for certain informalities;

(4) rejected claims 7-9 under 35 U.S.C. § 112 ¶ 1;

(5) rejected claims 7-9 under 35 U.S.C. § 112 ¶ 2; and

(6) rejected claims 7-9 under 35 U.S.C. § 102.

The Examiner has reminded Applicants of the necessity of a specific reference to an earlier application for the priority claim, along with a certified copy off the priority application. Applicants have amended the specification to include a specific reference to an earlier filed application, and include herein a certified copy of the foreign application, United Kingdom Application No. 9815283.8.

The Examiner stated that the sequence disclosure in Applicants' application failed to comply with definitions set forth in 37 C.F.R 1.821 through 1.825. Specifically,

the Examiner requested that the drawing descriptions on pages 25-26 and 29-31 of the specification be amended to refer to all represented sequences by SEQ ID NO. Applicants have so amended the specification, except for that of FIG. 2. Applicants urge that it is unnecessary to assign a SEQ ID NO. to the sequence of FIG. 2 because the sequence of enovin (hEVN) is disclosed in FIG. 1 and the remaining sequences depicted in FIG. 2 correspond to known proteins available in the databases. Applicants urge that this application now complies with the sequence requirements, and request examination under 35 U.S.C. §§ 131 and 132.

In addition, Applicants have amended the specification to include the heading, "Brief Description of the Drawings", on page 25, as suggested by the Examiner. Applicants thank the Examiner for this suggestion.

The Examiner objected to claim 7, requesting a comma be inserted between "encoding said growth factor or a functional equivalent". Applicants have deleted the phrase " or a functional equivalent", and thus urge that this objection is now moot. Reconsideration and withdrawal of this objection are respectfully requested.

The Examiner objected to claim 9 as being in improper dependent form. Claim 9 has been amended to depend from claim 8 instead of claim 7. Applicants urge that claim 9 further limits claim 8. Reconsideration and withdrawal of this objection are respectfully requested.

The § 112 Rejections

The Examiner rejected claims 7-9 under 35 U.S.C. § 112 ¶ 1 as containing subject matter not described or enabled in the written description. In particular, the Examiner stated that the specification failed to support or enable the phrase "or a functional equivalent, derivative or bioprecursor of said growth factor", that appears in each claim.

Applicants respectfully traverse these rejections.

Applicants have amended claims 7-9 to delete the phrase "or a functional equivalent, derivative or bioprecursor of said growth factor". Applicants urge that the amended claims 7-9 are supported and enabled by the specification. Reconsideration and withdrawal of these rejection are respectfully requested.

The Examiner rejected claims 7-9 under 35 U.S.C. § 112 ¶ 2 as being indefinite. The Examiner stated that because the claims recite a phrase, "or a functional equivalent, derivative or bioprecursor of said growth factor", that was not supported or enabled by the specification, the claims were indefinite.

Applicants respectfully traverse these rejections.

Applicants have amended claims 7-9 to delete the phrase "or a functional equivalent, derivative or bioprecursor of said growth factor". Because the amended claims are supported and enabled by the specification, as stated above, Applicants urge that the amended claims 7-9 are not indefinite. Reconsideration and withdrawal of these rejection are respectfully requested.

The § 102 Rejections

The Examiner rejected claims 7-9 under 35 U.S.C. § 102(a) as being anticipated by Baloh, *et al.*, Neuron 21:1291-1302, Dec. 1998.

Applicants respectfully traverse these rejections.

Applicants urge that the enclosed, certified copy of the priority document, United Kingdom Application No. 9815283.8, entitles Applicants to a priority date of July 14, 1998, which predates the December, 1998 publication date of the Baloh reference. Thus, the Baloh reference does not anticipate Applicants invention. Reconsideration and withdrawal of these rejection are respectfully requested.

The Examiner rejected claims 7-9 under 35 U.S.C. § 102(b) as being anticipated by Lin, *et al.*, Science 260:1130-1302, May 1993. The Examiner also rejected claims 7-9 under 35 U.S.C. § 102(e) as being anticipated by United States Patent No. 5,747,655 (Johnson, *et al.*).

Applicants respectfully traverse these rejections.

In order for a reference to anticipate under 35 U.S.C. § 102, the reference must disclose every claimed element of Applicants' invention, either expressly or under the principles of inherency.

Lin discloses a human GDNF precursor that shares a 28% similarity with Applicant's SEQ ID Nos. 3 and 4, and is thus a functional equivalent of said sequences. Johnson discloses a neurturin peptide that shares a 38.6% sequence similarity with Applicant's SEQ ID Nos. 3 and 4, and is thus a functional equivalent of enovin. However, Applicants have amended claims 7-9 to delete the phrase "or a functional equivalent, derivative or bioprecursor of said growth factor". Thus, both Lin and Johnson are directed to sequences not claimed by Applicants, and neither Lin nor Johnson discloses Applicants' SEQ ID Nos. 3 and 4, as claimed. Thus, neither Lin nor Johnson can anticipate Applicants' sequences. Reconsideration and withdrawal of these rejection are respectfully requested.

CONCLUSION

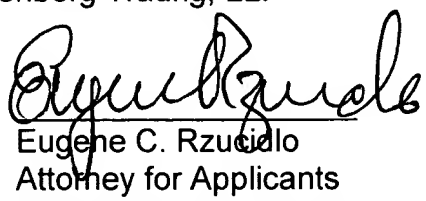
It is submitted that claims 7-9, as amended, are in condition for allowance. Early and favorable action by the Examiner is earnestly solicited. In addition, Applicants reserve the right to file a continuing or divisional application to further prosecute claims 17-18, 24, 41, 44, and 58-59, withdrawn from consideration by the Examiner. If the Examiner believes that issues may be resolved by a telephone interview, the Examiner is respectfully urged to telephone the undersigned at (212) 801-2146. The undersigned may also be contacted by e-mail at ecr@gtlaw.com.

AUTHORIZATION

The Commissioner is authorized to charge any required fees to the Greenberg Traurig Deposit Account 50-1561.

Respectfully submitted,
Greenberg Traurig, LLP

Date: March 25, 2002

By: 

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ATTACHMENT A

Amended Claims

7. An isolated human neurotrophic growth factor, designated enovin and having the amino acid sequence illustrated in Figure 1, known as SEQ ID No. 4, encoded by a nucleic acid molecule encoding said growth factor.

8. A growth factor according to claim 7 comprising the amino acid sequence from position 27 to 139 of the amino acid sequence illustrated in Figure 1, known as SEQ ID No. 3.

9. A growth factor according to claim 8 comprising the amino acid sequence illustrated in Figure 1, known as SEQ ID No. 4.

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NEUROTROPHIC GROWTH FACTOR

Cross References to Related Applications

The application claims benefit of priority under 35 U.S.C. § 119 from United Kingdom Patent Application No. 9815283.8, filed on July 14, 1998, the contents of which are incorporated herein by references, and is a continuation-in-part of United States Patent Application No. 09/327,668, filed on June 8, 1999, and of United States Patent Application No. 09/248,772, filed on February 12, 1999, the contents of which are incorporated herein by reference.

Field of the Invention

The present invention is concerned with a neurotrophic factor and, in particular, with cloning and expression of a novel member of the GDNF family of neurotrophic factors, designated herein as "enovin" (EVN).

Introduction

Neurotrophic factors are involved in neuronal differentiation, development and maintenance. These proteins can prevent degeneration and promote survival of different types of neuronal cells and are thus potential therapeutic agents for neurodegenerative diseases. Glial cell-line derived neurotrophic factor (GDNF) was the first member of a growing subfamily of neurotrophic factors structurally distinct from the neurotrophins. GDNF is a member of the transforming growth factor ß (TGF-ß) superfamily of growth factors, characterized by a specific pattern of seven highly conserved cysteine residues within the amino acid sequence (Kingsley, 1994) . GDNF was originally purified using an assay based on its ability to maintain the survival and function of embryonic ventral midbrain dopaminergic neurons in vitro (Lin et al., 1993). Other neuronal cell types in the central (CNS) or peripheral nervous systems (PNS) are also responsive to the survival effects of GDNF (Henderson et al., 1994, Buj-Bello et al., 1995, Mount et al., 1995, Oppenheim et al., 1995). GDNF is produced by cells in an inaction proform, which is cleaved

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As would be well known to those of skill in the art, the specific dosage regime may be calculated according to the body surface area of the patient or the volume of body space to be occupied, dependent upon the particular route of administration to be used. The amount of the composition actually administered will, however, be determined by a medical practitioner, based on the circumstances pertaining to the disorder to be treated, such as the severity of the symptoms, the composition to be administered, the age, weight, and response of the individual patient and the chosen route of administration.

Brief Description of the Drawings

The present invention may be more clearly understood by the following examples which are purely exemplary and by reference to the accompanying drawings wherein:

Figure 1: (SEQ ID NO. 2) is partial cDNA sequence of a neurotrophic factor according to the invention designated as enovin. The consensus sequence was obtained by PCR amplification with primers PNHsp3 and PNHap1 on different cDNAs and on genomic DNA followed by cloning and sequence analysis and comparison of the obtained sequences. The predicted one letter code amino acid sequence is shown above the DNA sequence. The nucleotide residue number is shown on the right of the DNA sequence, whereas the amino acid residue number is shown to the right of the translated protein sequence. The putative RXXR cleavage site for the prodomain is indicated in bold and underlined. The putative start of the mature protein is indicated by an arrow. The seven conserved cysteine residues characteristics for all members of the TGF-ß family

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are indicated in bold. A potential N-glycosylation site is double underlined.

Figure 2: is alignment of the predicted mature protein sequences of human GDNF, NTN, PSP and EVN. The sequences were aligned using the ClustalW alignment program. Amino acid residues conserved between all three proteins are included in the black areas. Residues conserved between two or three of the sequences are shaded in grey. The 7 conserved cysteine residues characteristic for members of the TGF-ß family are indicated by asterisks above the sequence. Amino acid residues are numbered to the right. The dashes indicate gaps introduced into the sequence to optimize the alignment.

Figure 3: (SEQ ID NO. 5) is partial cDNA sequence of enovin. The consensus sequence was obtained by PCR amplification (primary PCR with primers PNHsp1 and PNHap1 and nested PCR with primers PNHsp2 and PNHap2) on different cDNAs followed by cloning and sequence analysis and comparison of the obtained sequences. The translated one letter code amino acid sequence of nucleotides 30 to 284 (reading frame A) is shown above the sequence and numbered to the right (A1 to A85). This reading frame contains a putative ATG translation start codon. The translated one letter code amino acid sequence of nucleotides 334 to 810 (reading frame B) is shown above the sequence and numbered to the right (B1 to B159). This reading frame contains the region of homology with GDNF, NTN and PSP. The nucleotide residue number is shown to the right of the DNA sequence. The putative RXXR cleavage site for the

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Figure 20: is a graphic representation of the effects of enovin on taxol-induced sensory deficits using the pin prick test. Given are the average (± 1 SEM) cumulative scores over time of rats treated with either 2 different doses of enovin (23 or 130 µg/ml; n = 10 rats/ group) or vehicle / saline (n = 20 rats) before taxol. Enovin or saline / vehicle were injected in a volume of 75 µl in the subplantar area of the right hind paw.

Figure 21: (SEQ ID NO. 8) is a DNA sequence of enovin. The consensus sequence was obtained by amplification with PCR using primers PNHsp5 and PNHap1 on human frontal cortex cDNA and on human genomic DNA followed by cloning, sequence analysis and comparison of the resultant sequences. The predicted amino acid sequence is shown above the DNA sequence for the only splice variant yielding a functional Enovin protein after translation. The nucleotide residue number is shown to the left of the DNA sequence, whereas the amino acid residue number is shown to the right of the translated protein sequence. 5′ and 3′ splice sites detected by comparison of sequenced cDNA fragments with the genomic sequence are indicated by vertical lines bending to the left or right, respectively, and are numbered consecutively. The putative RXXR furin cleavage site for the prodomain is indicated in bold and underlined. The putative start of the mature protein is indicated by an arrow. The seven conserved cysteine residues characteristic for all members of the TGF-ß family are indicated in bold. A potential N-linked glycosylation site is double underlined. The 5′ and 3′ splice sites are numbered and encircled.

Figure 22: is an illustration of expression of different Enovin splice variants in human tissues. (A) schematic diagram of Enovin splice variants identified by RT-PCR experiments with Enovin specific primers on RNA derived from different human tissues followed by cloning and sequence analysis of PCR products. The top line shows a scale (in bp). The second line represents the Enovin genomic sequence. The position of the translation start and stop codon, of the start of the mature Enovin coding sequence and of the 5′ and 3′ splice sites (see Figure 21) are indicated. The right part of the figure shows the PCR products obtained by RT-PCR on ovary and on frontal cortex RNA together with a 100 bp DNA ladder. The position of the different mRNA variants is indicated together with their size (from start to stop codon). The translated proteins are shown on the left hand side. Boxes delineate regions represented in the cDNA. Dashed lines represent spliced out genomic DNA. The shaded region represents the mature Enovin coding sequence. The dotted line marks the start of the mature Enovin coding sequence. The two transcripts capable of yielding functional Enovin protein are indicated by an asterisk at the left hand side. (B) Tissue distribution of the main splice variants. The photograph shows the PCR fragments obtained by RT-PCR with Enovin specific primers on different human cDNAs. The 4 main splice variants (A to D) are indicated by arrows at the left hand side. Sizes are indicated on the right hand side based on the 100 bp DNA ladder used as size reference on the gel.

Figure 23: (SEQ ID NO. 9) Predicted protein sequence of the long

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splice variant of Enovin, obtained by splicing out the two introns from the DNA sequence of Figure 21. Splice sites 5'1 and 3'−1 are used to remove the first intron and splice sites 5'−2 and 3'−3 are used to remove the second intron. This results in a cDNA sequence having an open reading frame coding for the 228 amino acid residue protein shown above.

Figure 24: (SEQ ID NO. 10) Predicted protein sequence of an alternative (short) splice variant of Enovin, obtained by splicing out the two introns from the DNA sequence of Figure 21. Splice sites 5'−1 and 3'−2 are used to remove the first intron and splice sites 5'−2 and 3'-3 are used to remove the second intron. This results in a cDNA sequence having an open reading frame coding for the 220 amino acid residue protein shown above. This protein sequence misses 8 amino acid residues compared to the sequence of Figure 23.

Figure 25: is a graphic representation of the results obtained from experiments designed to compare the levels of expression of enovin in normal diseased tissue. Enovin and GAPDH expression is represented in brain tissue, in respect of multiple sclerosis and Alzheimer' s disease.

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Deposits

Plasmid EVNmat/pRSETB including the DNA sequence

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